REMARKS

It is believed that the currently proposed claims more clearly set forth the metes and bounds of the invention than those previously pending. No new matter has been added and entry of the substitute claims is respectfully requested.

As outlined in the specification, the invention is directed to a method to determine the activity of a protein that transfers a substance from donor to acceptor. The method corrects internally for varying or unknown amounts of acceptor in the test sample. In this way, samples with unknown concentrations of the transfer protein can be compared to a standard sample directly, without the necessity for other corrections to compensate for varying levels of acceptor.

The method comprises incubating a sample containing the transfer protein (which sample may contain an unknown concentration of acceptor) with a donor that contains the substance to be transferred. That substance is labeled with a light emitter wherein, in the donor supplied, the emission from the label is quenched. When the labeled substance to be transferred is released from the donor (and transferred to acceptor) the quenching no longer is effective and the fluorescence or luminescence of the sample increases. The activity of the transfer protein is thus reflected in an increase in emitted light.

However, the level of activity measured in the assay as described so far will increase with the concentration of acceptor present. The method of the invention is therefore also provided with a means to normalize for the concentration of acceptor. This is accomplished by generating an interference with the emitted light, such as color or turbidity, proportional to the level of acceptor. Thus, the intensity of light measured from emission due to the release of the substance from the donor is diminished proportional to the level of acceptor present, thus correcting for the acceptor

concentration. In this way, a reference sample assayed in the same way, can be used as a standard for comparison even if the reference sample contains a different level of acceptor.

The invention is illustrated by an assay for CETP which utilizes donor particles in which are contained cholesteryl ester or triglyceride coupled to the fluorescent label 7-nitrobenz-2-oxa-1,3-diazole (NBD) entrapped in particles such that the fluorescence of NBD is quenched. Further, the label remains in place in the presence of cholesteryl esterase, and the activity of the esterase is blocked. Such particles are known in the art as described in U.S. patents 5,585,235; 5,770,355 and 5,618,683 cited above. In general, these particles contain labeled cholesteryl ester or labeled triglyceride surrounded by phospholipids. The high concentration levels of the labeled lipids contained in the particles results in quenching of the fluorescence of the fluorophore. The donor particles are added to a sample in which the CETP activity is to be measured and incubated for sufficient time to effect transfer to any acceptor that may be present.

In prior versions of this assay, excess acceptor has been added to the biological sample to swamp out any effect of variability in the acceptor content endogenous to the sample. (The level of acceptor such as LDL and VLDL is highly variable among subjects to be tested as these levels are affected by diet. High saturated fat diets effectively raise the level of acceptors while fasting decreases these levels.) However, this requires an extra reagent, and the present invention method eliminates this need. In order to compensate for variations in level of acceptor, a normalizing color or turbidity is generated in proportion to the acceptor level.

The acceptor level is itself essentially proportional to unlabeled cholesteryl ester or triglycerides in the sample as the acceptors contain these moieties. Thus, reactions which generate

color or turbidity with respect to either of these moieties may be used as surrogates for acceptor in the normalizing reaction.

If cholesteryl ester is to be used as the index, a color is developed by, for example, providing a mixture of cholesteryl esterase, cholesterol oxidase, peroxidase and 4-aminoantipyrine and p-hydroxybenzenesulfonate in a buffer. Non-fluorescent cholesteryl esters are hydrolyzed to cholesterol (the cholesteryl-NBD is not) which is in turn oxidized to produce hydrogen peroxide which, in the presence of the detecting reagents, yields a visible dye. The visible dye absorbs some of the fluorescence emitted by the transferred cholesteryl ester labeled with NBD in proportion to the level of acceptor present.

Alternatively, triglyceride levels may be used as an index for the level of acceptor. This surrogate can be measured with a mixture of ATP, magnesium salt, 4-aminoantipyrine, sodium N-ethyl-N-(3-sulfopropyl)-m-anisidine, lipase, glycerol kinase, glycerol phosphate oxidase, peroxidase in buffer. This results in hydrolysis of the triglycerides to glycerol and free fatty acids; the glycerol is phosphorylated and then oxidized to generate hydrogen peroxide, which generates a color that absorbs the light emitted by the cholesteryl ester-NBD transferred to acceptor, where the level of color generated is proportional to the level of acceptor present.

It is believed that the currently proposed claims clearly reflect the invention. However, if further clarification is required, a telephone call to the undersigned is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 527832000300.

Respectfully submitted,

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